ALTERNATIVE PATHWAY OF COMPLEMENT:
DEMONSTRATION AND CHARACTERIZATION OF INITIATING
FACTOR AND ITS PROPERDIN-INDEPENDENT FUNCTION*.
‡

BY ROBERT D. SCHREIBER,§ OTTO GÖTZE,¶ AND HANS J. MÜLLER-EBERHARD*†

(From the Department of Molecular Immunology, Scripps Clinic and Research Foundation,
La Jolla, California 92037)

In 1954 Pillemer and associates (2) introduced the properdin system as a
humoral mechanism of natural resistance to infections. Although this provocac-
tive hypothesis generated a large body of literature, it could not be critically
evaluated until recently. The past 5 yr of complement research brought to light
an unanticipated complexity of the properdin system. The design of a rational
model of the properdin pathway had to await identification of the major compo-
nents and insight into their interactions.

In this communication and subsequent papers (3 and footnote 1), we wish to
report the results of experiments that together with previously published obser-
vations have allowed the formulation of a comprehensive molecular concept of
the properdin pathway (4). The dynamics of the pathway involve the recruit-
ment of the initiating factor (IF), sequential formation of C3 and C5 conver-
tases, activation of properdin, and stabilization of the enzymes by activated
properdin. The biological consequences of this process are identical to those
resulting from activation of the classical pathway: generation of the anaphylatoxins,
immune adherence reactivity, opsonic activity, and formation of the membrane attack complex.

The purpose of this paper is to describe (a) the initiating factor as a novel
serum protein, (b) its function in the assembly of the initial C3 convertase of the

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Abbreviations used in this paper: C3bINA, C3b inactivator; CVF, cobra venom factor; FITC,
flourescein isothiocyanate; GVBE, VB containing 0.1% gelatin and 0.04 M EDTA; IF, initiating
factor; IF, acid-treated IF: Mg-GVB, VB containing 0.1% gelatin and 1.3 x 10^-3 M MgSO4; NF,
nephritic factor; SDS, sodium dodecyl sulfate; VB, isotonic Veronal-buffer saline, pH 7.4; VB++,
VB containing 1.5 x 10^-1 M CaCl2 and 5 x 10^-1 M MgCl2.
pathway, and (c) the ability of the entire pathway to function in the absence of properdin.

Materials and Methods

Purified Components. Highly purified C3 (5), Factor B (6), Factor D (7), activated properdin (8), C4 (9), C5 (5), and cobra venom factor (CVF) (10) were isolated by previously reported methods.

Buffers. VB: isotonic Veronal-buffered saline, pH 7.4. VB+: VB containing 1.5 × 10⁻⁴ M CaCl₂ and 5 × 10⁻⁴ M MgCl₂. Mg-GVB: VB containing 0.1% gelatin and 1.3 × 10⁻³ M MgSO₄. GVBE: VB containing 0.1% gelatin and 0.04 M EDTA.

Preparation of Immune Adsorbents. The IgG fractions of various antisera were covalently coupled to CNBr-Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) utilizing the method of March et al. (11). The antisera to properdin and Factor D were raised in rabbits, the antisera to Factor B, C4, and nephritic factor (NF) were raised in goats. NF was purified as described (12), subjected to polyacrylamide gel electrophoresis, and the NF activity containing eluates from gel segments were used for immunization.

Immune Adsorption of Normal Human Serum. Fresh normal human serum was immunochemically depleted of various components by the method described previously (12). Recovery of material specifically bound to the immune adsorbent was accomplished by elution with 0.2 M glycine HCl buffer, pH 2.2, and collection of fractions into an equal volume of 0.2 M Na₂HPO₄. The eluate was dialyzed against phosphate-buffer saline and concentrated.

Zymosan or Inulin Tests. The ability of zymosan or inulin to activate the alternative pathway in the various depleted sera was assessed by addition of 250 μg of activator to 50 μl of serum in a final vol of 70 μl and a final concentration of magnesium ions of 1.2 × 10⁻³ M. After incubation for 30 or 60 min at 37°C, the reaction was stopped by addition of GVBE and lowering the temperature to 0°C. C3 consumption was determined by effective molecule titrations as described elsewhere (13).

Immunofluorescence. The detection of surface bound C3 and properdin was accomplished using the direct immunofluorescence technique employing (FITC) fluorescein isothiocyanate-conjugated antibody prepared according to the method of Clark and Shepard (14). The antiserum to C3 was obtained by immunizing rabbits with the isolated protein. The antiserum was specific for the c and the d portion of C3b.

Electrophoresis. Disk electrophoresis was performed using the method of Davis (15) in 6% running gels and Tris-HCl-glycine buffer, pH 8.9. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed according to the method of Weber and Osborn (16) as reported previously (9). Analytical agarose block electrophoresis was carried out on 5 × 7.6-cm glass slides containing 10 ml of 1.2% agarose in 0.044 M barbital-HCl buffer, pH 8.6, containing 0.01 M EDTA for 2 h at 5.3 V/cm.

Sucrose Density Gradient Ultracentrifugation. This was performed in 5-ml linear 7-31% sucrose density gradients in VB+ at 40,000 rpm for 16 h at 4°C in a Beckman L5-65 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). Markers employed were IgG (6.6S), HSA (4.5S), and cytochrome c (1.7S).

Partial Purification of IF. The serum of 2 U of freshly drawn normal human blood was dialyzed against 3 × 10 liters of 0.005 M phosphate buffer, pH 7.3. The precipitate that formed was removed by centrifugation, and the pseudoglobulin fraction was applied to a 6 x 80-cm column of DEAE cellulose equilibrated with the same buffer. The column was washed with 3 liters of starting buffer, and IF was eluted with a 6-liter linear NaCl concentration gradient, utilizing as limit buffer 0.005 M phosphate buffer, pH 7.3, containing 0.4 M NaCl. IF-containing fractions were pooled, concentrated to 15 ml, and subjected to molecular sieve chromatography on a 5 × 100-cm column of Bio-Gel A-0.5 M equilibrated with 0.01 M phosphate buffer, pH 7.3, containing 0.14 M NaCl. After concentration, the IF-containing pool was passed through an immune adsorbent column containing antibody to IF and C3b inactivator (C3bINA). After washing, IF and C3bINA were eluted with 0.2 M glycine-HCl buffer, pH 2.2. The eluted material was neutralized, dialyzed, and concentrated. IF and C3bINA could be separated by electrophoresis on 6% polyacrylamide gels at pH 8.9. The gels were cut into 2-mm segments which were eluted with 200 μl of Veronal-buffered saline. IF activity was measured by its ability to reconstitute IF-depleted serum with respect
to C3 consumption by zymosan. C3 consumption was quantitated as indicated above. C3bINA was measured by its ability to inactivate the classical C5 convertase on EAC4,°xY2,3. 1.5 x 10⁷ assay cells were incubated for 10 min at 37°C with 20 µl of eluted material in a total vol of 30 µl. The cells were then washed and incubated with 1 ml of C5-9 reagent to determine the reduction in C5 convertase activity.

Lysis of Rabbit Erythrocytes. Rabbit erythrocytes were collected from freshly drawn EDTA blood, washed four times with GVBE, then three times with Mg-GVB. Cells were standardized to a final concentration of 1.5 x 10⁹/ml. Reaction mixtures for alternative pathway-mediated lysis of rabbit erythrocytes contained 100 µl Mg-GVB, 100 µl rabbit erythrocytes (at 1.5 x 10⁶ erythrocytes/ml), and 100 µl of serum usually diluted 1:5 in Mg-GVB. To ensure no contribution of the classical pathway, all sera were immunochemically depleted of C4. Additionally, in some experiments, the sera were adsorbed three times at 1°C with rabbit erythrocytes in the presence of EDTA. After incubation of the reaction mixtures at 37°C for 30 min, the reaction was stopped by addition of 1 ml of cold GVBE. The remaining cells were pelleted and hemolysis determined by optical density measurements at 412 nm.

Results

The Initial C3 Convertase: A Properdin-Independent Enzyme. To examine the possible role of properdin in the initiation of the pathway, serum was immunochemically depleted of properdin. To eliminate interference by the classical pathway this and all other serum reagents were immunochemically depleted also of C4. Particulate inulin or zymosan were employed as characteristic activators of the properdin system. In absence of properdin, significant C3 consumption and deposition of C3 on the activator particles was observed. C3 activation was dependent on the presence of Factors B and D and magnesium ions (Mg), as evidenced by abrogation of C3 utilization by further depletion of the serum reagent of either Factors B, D, or Mg (Table I). It is concluded from these results that serum allows the generation of an alternative C3 convertase in absence of properdin. The function of this enzyme is to deposit C3b on the surface of the activating particle, where it serves as the receptor for activated properdin, as was shown previously (12). That bound C3b constitutes the only properdin receptor is indicated by negative properdin deposition on zymosan upon incubation with C3-deficient serum.

Recognition of the IF. In contrast to the above results, no C3 consumption or deposition were observed when zymosan was incubated with serum previously treated with solidified antibody to NF (Table I). The negative result was obtained in spite of the fact that the anti-NF-treated serum contained undiminished concentrations of properdin, C3, Factors B and D, and sustained C3 consumption on addition of NF, activated properdin (P), or CVF (Table II).

Proteins specifically retained by the anti-NF immune adsorbent were eluted with acid buffer, dialyzed, and tested for the ability to reconstitute the anti-NF-treated serum. As shown in Table I, addition of a portion of the eluate to the treated serum restored C3 consumption and C3 and properdin deposition in the presence of zymosan. In this Table the activity is referred to as IF to distinguish it from NF activity which has not been found in normal serum. When properdin was removed from IF-depleted serum and the serum was restored with respect to IF activity, significant C3 binding to zymosan was observed which, however, was lower than in properdin-containing serum.

That IF was recovered from the immune adsorbent in precursor form is demonstrated in Fig. 1. Consumption of C3 in IF-depleted serum was negligible.
**R. D. SCHREIBER, O. GÖTZE, AND H. J. MÜLLER-EBERHARD**

### Table I

**Initiation in Absence of Properdin(P): Requirement of Initiating Factor, Factors B, D, and Native C3 for Formation of the Properdin Receptor-Forming Enzyme**

<table>
<thead>
<tr>
<th>C4-deficient serum depleted of</th>
<th>Material added</th>
<th>C3 consumption* + Zymosan + NaCl</th>
<th>Deposition on zymosan</th>
<th>Immunofluorescence*</th>
<th>C3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>cpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>22.4</td>
<td>6.9</td>
<td>ND</td>
<td>+2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P, B</td>
<td>2.8</td>
<td>1.8</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P, D</td>
<td>0.6</td>
<td>4.1</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Me++ (EDTA)</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>54.6</td>
<td>4.5</td>
<td>237</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IF, IF</td>
<td>10.1</td>
<td>10.0</td>
<td>210</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P, IF</td>
<td>IF</td>
<td>54.6</td>
<td>6,423</td>
<td>+4</td>
<td>+4</td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>C4</td>
<td>76.6</td>
<td>2,871</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* Reaction mixtures containing 50 µl serum, 250 µg zymosan, 10^{-5} M Mg++ incubated 30 or 60 min at 37°C. C3 consumption determined by effective molecule titration. Zymosan particles were washed and incubated 45 min with the FITC-conjugated IgG fraction of monospecific antisera to C3 or P.

### Table II

**Unchanged Concentrations of Four Components of the Properdin(P) System in IF-Depleted Serum**

<table>
<thead>
<tr>
<th>C4-deficient serum depleted of</th>
<th>Concentration* C3§ B§ D</th>
<th></th>
<th>Zymosan</th>
<th>P</th>
<th>NF</th>
<th>CVF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>&gt;90</td>
<td>100</td>
<td>105</td>
<td>100</td>
<td>0</td>
<td>99.8</td>
</tr>
<tr>
<td>Nothing</td>
<td>&gt;90</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>56</td>
<td>98.4</td>
</tr>
</tbody>
</table>

* Percent of concentration in the untreated serum.

### On addition of IF alone, however, it approached completion in presence of zymosan.

The experiments described so far indicate that the initial C3 convertase is generated from IF and Factors B and D in the presence of Mg. Because of its function, IF was given the trivial name initiating factor.

To clearly establish the sequence of action of IF and properdin, a transfer
FIG. 1. Reconstitution of IF-depleted serum by IF-purified immunochemically in precursor form. This is demonstrated by zymosan-dependent consumption of C3 as a function of IF. 50 μl IF-depleted serum was incubated with varying amounts of IF in presence or absence of zymosan for 30 min at 37°C. C3 consumption determined by effective molecule titration.

TABLE III

<table>
<thead>
<tr>
<th>First treatment* with</th>
<th>Result of first treatment</th>
<th>Result of second treatment† with IF-depleted HS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Consumption C3</td>
<td>Deposition C3</td>
</tr>
<tr>
<td>P-depleted HS</td>
<td>22.4</td>
<td>2+</td>
</tr>
<tr>
<td>C3-deficient HS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NaCl</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Zymosan treated for 60 min at 37°C with reagent, washed with GVBE, kept for 2 h at 22°C and for 16 h at 4°C.
† Treated for 30 min at 37°C with reagent.

experiment was performed. Zymosan was first incubated in properdin-depleted serum, washed extensively, and then incubated in IF-depleted serum. Table III shows that during the first incubation C3 was deposited on the particles in absence of properdin. During the second incubation, properdin was deposited in absence of IF and C3 consumption and uptake increased. This experiment not only establishes the sequence of action, but together with the above results, shows that C3 deposition, as affected by the IF-dependent C3 convertase, is a prerequisite for properdin binding. This enzyme therefore may be called the properdin receptor-forming enzyme.

Partial Purification and Characterization of the IF. Fig. 2 shows that the unactivated form of IF in serum migrates as a β-globulin upon electrophoresis at pH 8.6 in 1% agarose gel. Fig. 3 demonstrates the behavior of IF on molecular sieve chromatography using Sephadex G-150. Its apparent filtration rate indicates a mol wt of 70,000–120,000 daltons.

Partial purification was achieved by a four-step procedure. Since IF was found to be a pseudoglobulin, the pseudoglobulin fraction of normal human serum (400 ml) was prepared and subjected to anion exchange chromatography using DEAE
FIG. 2. Electrophoretic migration of IF as a β-globulin in serum. 75 µl of C4- and properdin-depleted human serum containing 0.01 M EDTA was subjected to electrophoresis in a 5 × 7.6-cm agarose slab at pH 8.6. After sectioning into 3-mm segments, the slices were eluted with 100 µl VB. IF activity was determined by reconstitution of zymosan-dependent C3 consumption in IF-depleted serum utilizing 25 µl of the segment eluates, both untreated or heated to 56°C for 30 min.

FIG. 3. IF exhibits an apparent mol wt of 70,000-120,000 daltons upon molecular sieve chromatography on Sephadex G-150. 10 ml of C4 and properdin-depleted serum containing 0.01 M EDTA applied to a 5 × 100-cm Sephadex G-150 column equilibrated with phosphate-buffer saline containing 2 mM EDTA at a flow rate of 50 ml/h. IF activity of pools determined as in Fig. 2.

cellulose at pH 7.4 and a NaCl concentration gradient elution procedure. IF was eluted at 5.4 mmho/cm together with transferrin and C3b inactivator (Fig. 4). The active fractions (No. 60-68) were pooled, concentrated, and applied to a Bio-Gel A-0.5 M column equilibrated with phosphate-buffered saline, pH 7.3. The IF was eluted as a 70,000-120,000-dalton protein, and the elution pattern was
ALTERNATIVE PATHWAY OF COMPLEMENT

Fig. 4. DEAE chromatography of pseudoglobulin containing IF. 400 ml of the pseudoglobulin fraction of normal human serum was applied to a 6 x 80-cm column of DEAE cellulose equilibrated in 5 mM phosphate buffer, pH 7.3. The column was washed until the breakthrough had eluted, after which a NaCl concentration gradient was started at tube no. 1. The protein was eluted together with transferrin and C3b inactivator.

similar to that depicted in Fig. 3. The IF-containing material was then passed over an immune adsorbent column with specificity for IF and C3b inactivator. Elution of unactivated IF and C3b inactivator was accomplished with 0.2 M glycine-HCl buffer, pH 2.2. The particular antiserum was chosen because it is the most potent anti-IF antiserum tested and because monospecific antiserum to IF is not yet available.

Direct demonstration of IF as a distinct protein was possible by alkaline polyacrylamide gel electrophoresis. Fig. 5 shows the separation of IF from C3b inactivator and the identification of both proteins on the basis of their distinct activities.

The sedimentation velocity of IF upon sucrose density gradient ultracentrifugation was found to be 7S (Fig. 6), which is similar to that of NF (18). This value suggests a higher molecular weight than indicated by the results of the molecular sieve chromatography experiments. By polyacrylamide gel electrophoresis in presence of SDS, the mol wt of IF was found to be slightly greater than that of IgG, approximately 170,000. After reduction of IF with dithiothreitol in 8 M urea and 1% SDS the protein exhibited a mol wt of 85,000 (Fig. 7). This result indicates that IF is composed of two probably identical polypeptide chains which are linked by disulfide bonds. IF therefore possesses a polypeptide chain structure that is indistinguishable from that of NF (19, 20).

Whether a physiologically activated form of IF exists that is analogous to NF is not known. However, NF-like activity (12, 19-21) was generated by treatment of IF with 0.2 M glycine-HCl buffer, pH 2.2, for 5 h at 4°C. Acid-treated IF (IF) led to C3 consumption in IF- and properdin-depleted serum in absence of zymosan, stabilized the cell-bound labile C3 convertase, C3b,B and caused agglutination of EC3b only in presence of Factors B, D, and Mg (Table IV). Unlike unactivated IF, IF migrated as a γ-globulin upon agarose gel electrophoresis at pH 8.6 (Fig. 8). In this regard, too, it resembles NF.

Lysis of Rabbit Erythrocytes Via the Alternative Pathway Requires IF but not Properdin. Because properdin was found to be unessential for initiation, we
explored its requirement for the cytolytic effect of the properdin pathway. Platts-Mills and Ishizaka (22) have shown that rabbit erythrocytes are unusual in that in human serum they undergo lysis due to their ability to activate the alternative pathway. Using this system, it was found that properdin-depleted serum sustained lysis of the cells, although to a lesser extent than serum containing properdin. Lysis was virtually abolished after removal of the IF from the serum (Fig. 9). The hemolytic capacity of the IF-depleted serum was recovered in a dose-related fashion upon addition of partially purified IF (Fig. 10).
ALTERNATIVE PATHWAY OF COMPLEMENT

Fig. 7. Molecular weight (MW) determination of reduced IF by SDS polyacrylamide gel electrophoresis in 7% polyacrylamide gels. A value of 85,000 daltons was obtained as compared to 170,000 daltons for unreduced IF as determined by the same method (not shown). Markers: Phos a (phosphorylase a), 100,000; Fib a, b, g (Fibrinogen a, b, and g chains), 70,000, 60,000, and 50,000, respectively; G6P Dehyd (glucose-6-phosphate dehydrogenase), 36,000; and chy (chymotrypsinogen), 25,000.

Table IV

<table>
<thead>
<tr>
<th>EC3b incubated with</th>
<th>Stabilized C3/C5 convertase (Z)</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF, B, D*</td>
<td>2.0</td>
<td>4+</td>
</tr>
<tr>
<td>NF, B, D*</td>
<td>2.2</td>
<td>4+</td>
</tr>
<tr>
<td>IF, B, D*</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>B, D*</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>IF, wash, B, D†</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>NF, wash, B, D†</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Buffer*</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Z, 63% lysis.

* Reaction mixtures containing 10⁸ EC3b, 1 μg B, 13.5 ng D, with or without 20 μl IF, NF, or IF and 10⁻³ M Mg in a total vol of 70 μl, incubated 10 min, 37°C, washed with GVB, decayed 10 min at 37°C, and then mixed with 1 ml of C3-9 reagent.† First incubation: 10⁸ EC3b, 20 μl IF, or NF and 10⁻³ M Mg for 10 min, 37°C, washed with Mg GVB. Second incubation: 10 min, 37°C, with 1 μg B, 13.5 ng D, and 10⁻³ M Mg in a total vol of 70 μl. Cells were washed in GVB, decayed 10 min, 37°C, then mixed with 1 ml C3-9 reagent.

Discussion

This is the first description of the IF of the properdin system. To our knowledge the protein has been heretofore unrecognized with respect to both its function and its physicochemical properties. IF is a 170,000 dalton pseudoglobulin with a sedimentation coefficient of 7S and the electrophoretic mobility of a β-globulin. It is composed of two apparently identical polypeptide chains which are linked by disulfide bridges and noncovalent forces. Its biological activity is thermostable. The discrepancy encountered with molecular weight estimates using Sephadex filtration and SDS polyacrylamide gel electrophoresis suggest that IF is retarded on Sephadex filtration due to interaction with the polysaccharide.
Electrophoretic behavior of IF "activated" by treatment at low pH. IF migrates as a γ-globulin at pH 8.6. 75 μl of partially purified IF containing Ig as contaminant was subjected to electrophoresis in a 5 × 7.6-cm agarose slab. After sectioning into 3-mm segments and elution with VB, IF activity was determined by the ability of the eluates to induce consumption of C3 in IF-depleted serum in absence of zymosan.

Initiating factor-dependent lysis of rabbit erythrocytes in presence or absence of properdin. Varying amounts of C4, C4 and properdin, or C4 and IF-depleted serum were incubated for 30 min at 37°C with 1.5 × 10⁷ rabbit erythrocytes in a total vol of 150 μl. Reactions were stopped by dilution with 1 ml cold GVBE and centrifugation.

Alternative pathway dependent lysis of rabbit erythrocytes as a function of dose of IF in IF-depleted serum. 1.5 × 10⁷ rabbit erythrocytes were incubated with 20 μl IF-depleted serum and varying doses of partially purified IF as described in Materials and Methods.

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Some properties of IF resemble those of coagulation Factor XI (23). This factor is a relatively heat stable protein and has a mol wt of 160,000. It is composed of two probably identical 80,000-dalton chains which are linked by disulfide bonds. Unlike IF, Factor XI is a zymogen which on activation is cleaved into 50,000 and 33,000 dalton fragments. The enzyme and the zymogen have the electrophoretic mobility of γ-globulin. A relationship of IF to Factor XI is therefore considered unlikely.

The function of IF is thought to be twofold: we assume that IF represents the recognition unit of the alternative pathway and we have presented evidence that it generates the initial C3 convertase. That IF may indeed be the recognition unit is suggested by the experiments utilizing IF-depleted serum. Although all known components of the properdin system were present, the system was unresponsive to all genuine alternative pathway activators tested: zymosan, inulin, rabbit erythrocytes, and antibody-coated measles virus-infected cells (unpublished observations). The recognition unit of the alternative pathway must be able to react with a variety of disparate substances, including fungal and bacterial cell wall constituents (24), certain animal cell membrane constituents (22, 25), aggregates of a limited number of immunoglobulins (26, 27), budding virus-infected cells coated with anti-virus antibody (28, 29), and chemically defined substances such as benzyl-β-D-fructopyranoside (30), polyglucose with consecutive α-1.3 and branched α-1.6 linkages (31), and dinitrophenylated albumin (32). It will be the task of future investigations to determine the common chemical or physicochemical denominator of these substances in regard to their activator function. If IF is the recognition unit of the pathway, it would have to be able to recognize all of them. Certainly, purified IF is able to restore the function of IF-depleted serum with respect to any of the known activators of the pathway. The possibility cannot be excluded, however, that IF represents a group of closely related proteins which differ in recognition specificity.

Its function as nucleus of the initial C3 convertase is obviously dependent on some kind of physical interaction with an activator substance. This postulated interaction appears to confer a new property upon IF, the ability to bind to Factor B and to C3 and to generate a C3 convertase with Factor D and Mg. That IF is potentially capable of undergoing a conversion to an "activated" form was suggested by the activity expressed by acid-treated IF. That the "activated" form of IF exhibits an affinity primarily for activated Factor B was documented by the dependence of agglutination of cells bearing C3b on the presence of activated Factor B. The entity to which "activated" IF attaches is the cell-bound complex C3b,B, not cell-bound C3b.

By analogy to the NF-dependent C3 convertase (12), we propose that the physiologically activated IF utilizes not only Factors B and D, but also native C3 to form the initial C3 convertase according to the following expression:

\[
\text{IF} + \text{C3} + \text{B} + \text{D} \xrightarrow{\text{Activator}} \text{Mg} \xrightarrow{\text{C3,B,IF}}
\]

Whether Factor D is an integral subunit of the enzyme complex is not known.

As demonstrated in this paper, the biological function of this enzyme is to activate C3 and to deposit C3b on the surface of the activating particle, where it serves as a subunit and anchor of the subsequently formed particle bound
enzymes, the properdin activating principle, and finally as the receptor of activated properdin itself (3).

The precise relationship of IF to NF is unknown. That antisera to NF react with IF is interpreted to indicate close immunological relatedness or identity of the two proteins. The first indication of the relationship of NF to a normal serum constituent which functions in the properdin system was reported from our laboratory in 1974 (18). The virtual identity of IF and NF (19, 20) with respect to molecular weight and chain structure lends further support to their similarity. The two proteins differ, however, in electrophoretic mobility and apparent biological activity. Exploratory experiments with acid-treated IF show that an IF derivative may be produced in vitro that resembles NF in both biological activity and electrophoretic mobility. Like NF, acid-treated IF behaved like a γ-globulin upon electrophoresis, affixed itself to EC3b,B, stabilized this cell-bound enzyme, and caused agglutination of this cell-enzyme complex. A valid evaluation of NF will have to await a full understanding of IF.

The difference in function of IF and properdin is fundamental. IF is operative in the earliest events of the properdin pathway, properdin is not. IF interacts primarily with activated Factor B, properdin interacts primarily with C3b. IF appears to be essential for the entire sequence as are Factors B, D, and C3 but properdin is not strictly an essential component as evidenced, for instance, by the IF-dependent lysis of rabbit erythrocytes in properdin-depleted serum. Other cellular systems seem to require properdin for detectable cytolysis. Measles virus-infected cells coated with antibody to the virus are lysed by complement via the alternative pathway. This reaction requires all components, including IF and properdin (unpublished observations). Another example of the properdin-independent function of the pathway is the cold-dependent activation of C3 in nephritic serum (33). This reaction proceeds entirely via the alternative pathway and requires Factors B and D, but not properdin.

With the identification of IF as an essential, early acting component of the alternative pathway, it has been possible to delineate the physiological sequence of events of the properdin pathway, including the recruitment of precursor properdin and the functions of the recruited protein. Experiments pertaining to events after the IF engagement will be reported separately (3 and footnote 1).

Summary

A novel component of the properdin system has been described which represents a heretofore unrecognized human serum protein. The protein has been tentatively termed the initiating factor (IF) because it functions in the initial reaction of the properdin pathway. IF is a 170,000 dalton β-pseudoglobulin which is composed of two presumably identical 85,000 dalton chains linked by disulfide bonds. The protein reacts with antibody to nephritic factor, which is defined by its activity and is found in the serum of patients with certain nephritides.

The activity of IF is heat stable. Upon treatment of serum with activators of the alternative pathway, the initial C3 convertase is assembled from IF, Factors B and D, C3, and magnesium without participation of properdin. It is the function of the enzyme to deposit C3b on the surface of the activator particles,
ALTERNATIVE PATHWAY OF COMPLEMENT

thereby affording generation of the solid phase enzymes of the pathway, a process that is a prerequisite for properdin activation.

By exposure to low pH, IF assumed the electrophoretic mobility of γ-globulin and acquired the ability to generate without activators a fluid phase C3 convertase in serum. Serum depleted of IF did not allow activation of the properdin pathway. Serum depleted of properdin did permit activation of the pathway and expression of cytolytic activity. These results raise the possibility that IF represents the recognition unit of the pathway.

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R. D. SCHREIBER, O. GÖTZE, AND H. J. MÜLLER-EBERHARD


